Course 27820 - Medical Biofilm Techniques

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Microbial Diagnostics Exercise

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Microbial diagnostics: Identification and characterization of bacteria in a medical scenario

Learning objectives:

A student who has met the objectives of the course will be able to:

- Perform biofilm flow cell setup and operation: Manipulation and monitoring of growing biofilms, antibiotic treatment of biofilms, viability assessment, on-line measurements of oxygen in biofilms, harvesting of biofilms.
- Perform biofilm micro titer tray assays.
- Use advanced optical microscopy techniques: Confocal microscopy and fluorescence microscopy, time-lapse (confocal) microscopy.
- Use atomic force microscopy (AFM) for nano scale imaging.
- Use flow cytometry for single cell analysis.
- Understand chip technology as a tool for gene analysis.
- Perform mathematical image processing using COMSTAT analysis.
- Perform digital image preparation and presentation (Imaris).
- Present biofilm experiment results for an audience.

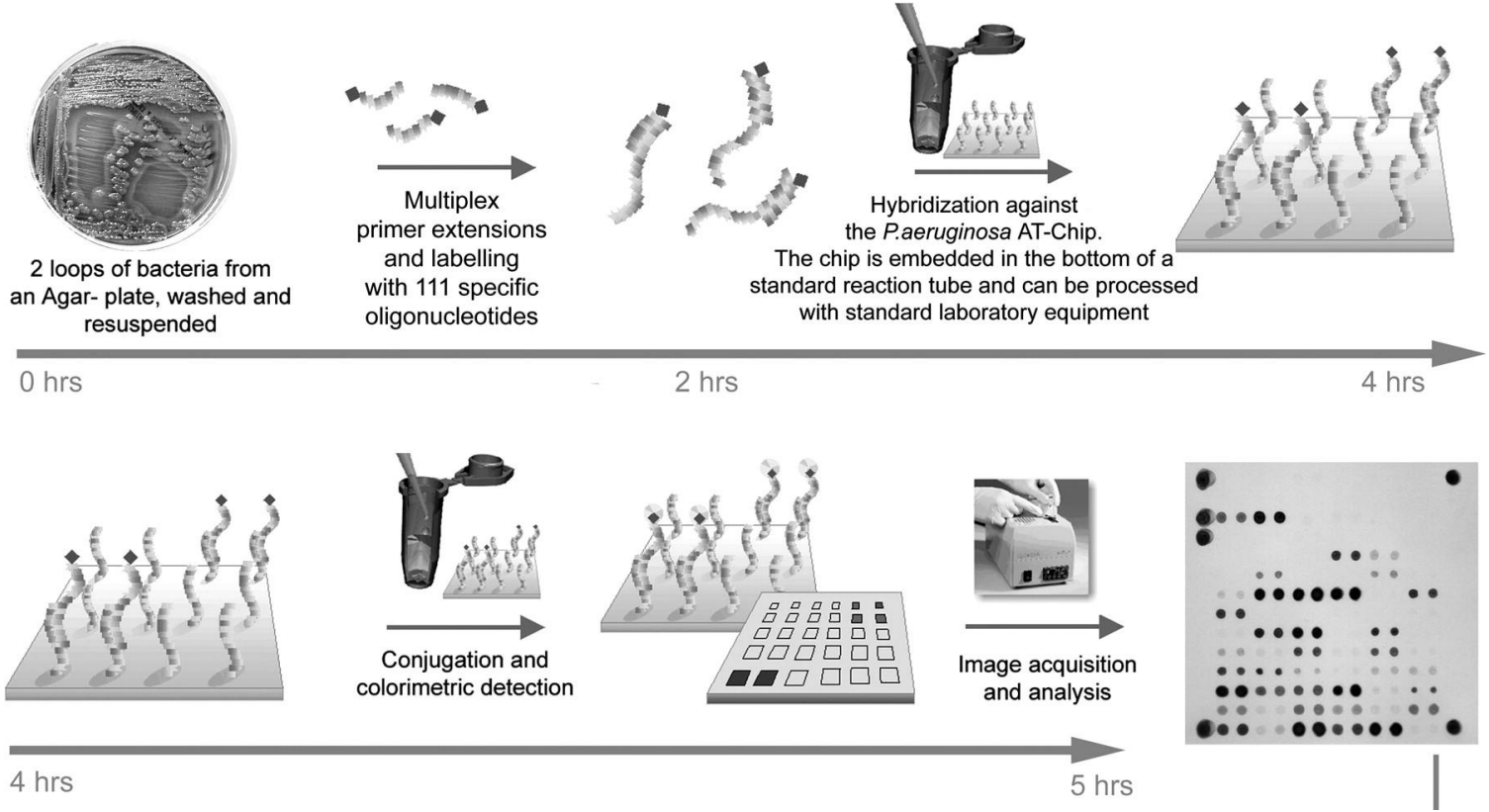
Genetic typing methods...

Outline of the ArrayTube Chip from Clondiag Chip Technologies GmbH

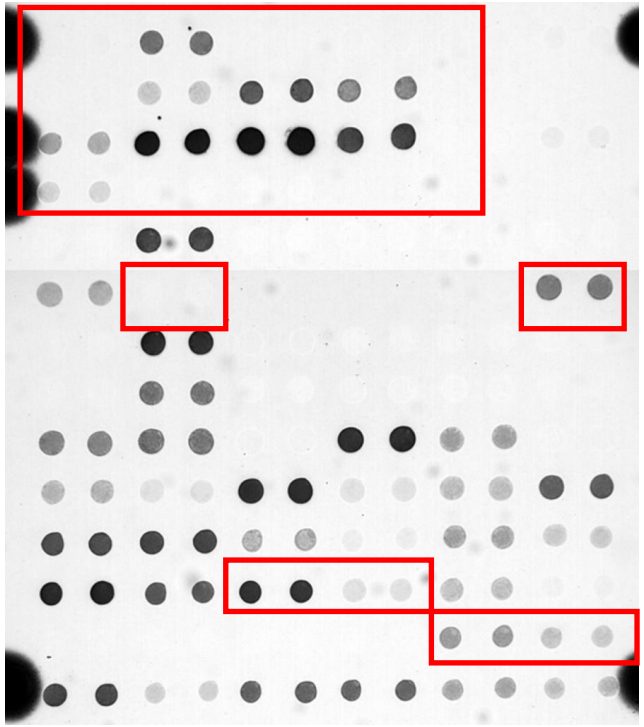
Gene islands and variable genes (*e.g.* pathogenicity factors such as *exoS* and *exoU)*

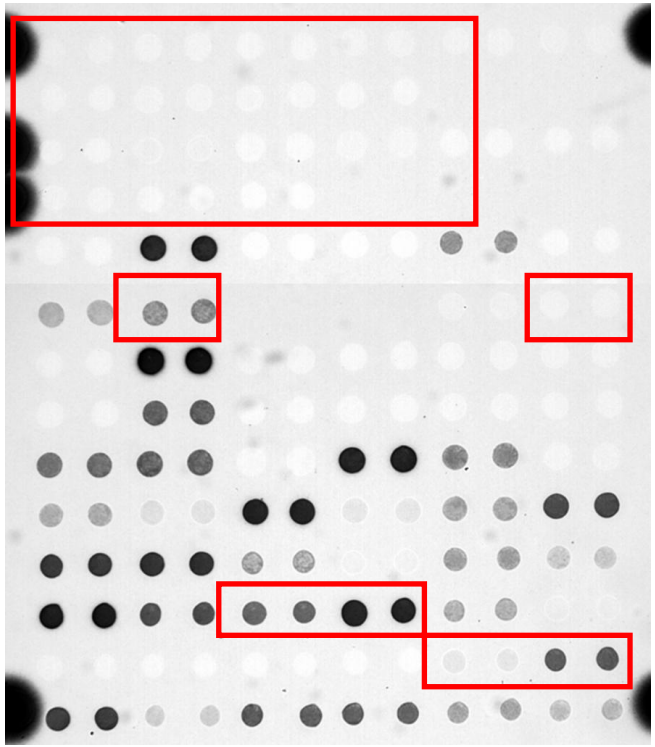
SNPs within conserved genes

Flow chart of *P. aeruginosa* genotyping with the low-resolution microarray



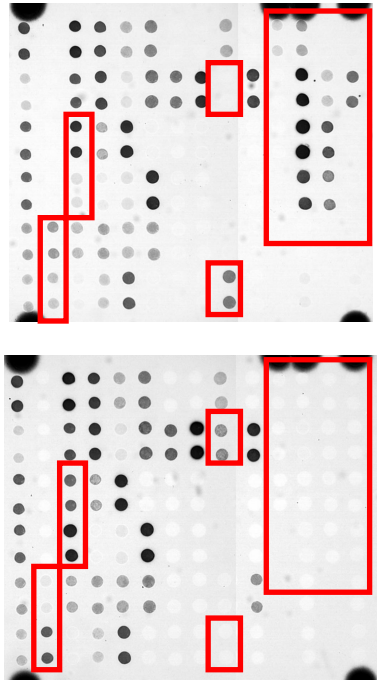
The AT chip is able to discriminate different clone types of *P. aeruginosa*



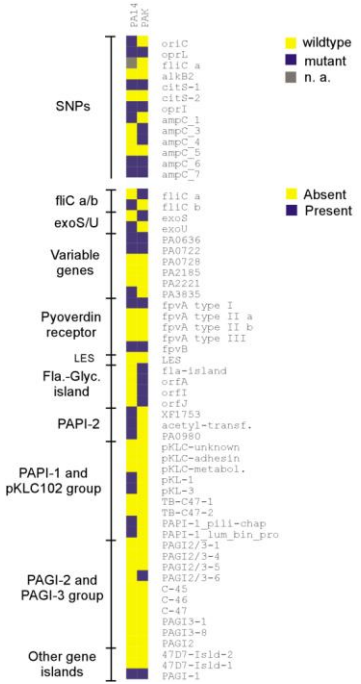


Detection of specific SNPs and presence/absense of genes measures the genotype which can be easily compared and used to define clone types

Chip-images



Genotype information



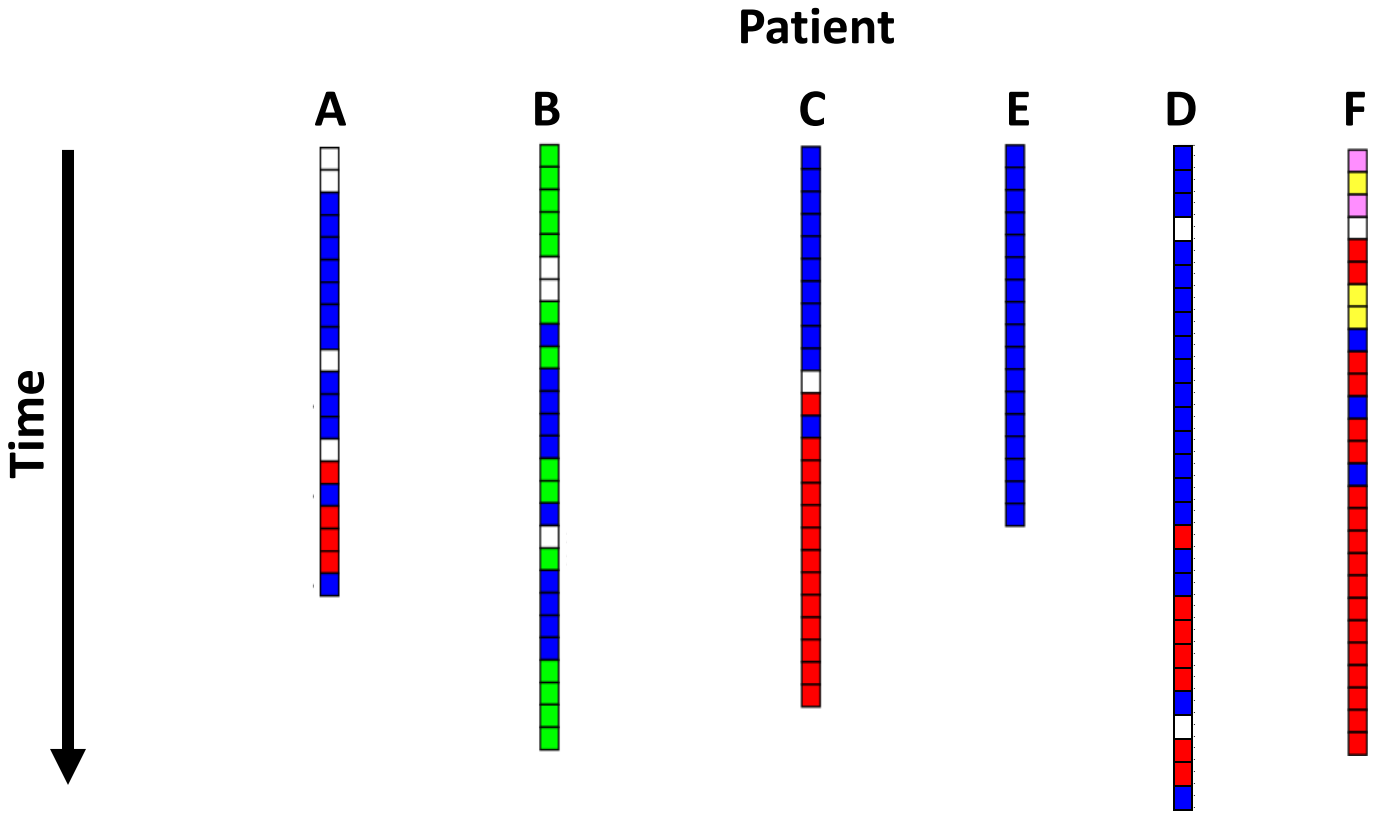
Clone types

DK1

DK2

Motivating Case story – Molecular evolution of *Pseudomonas aeruginosa* in cystic fibrosis patients

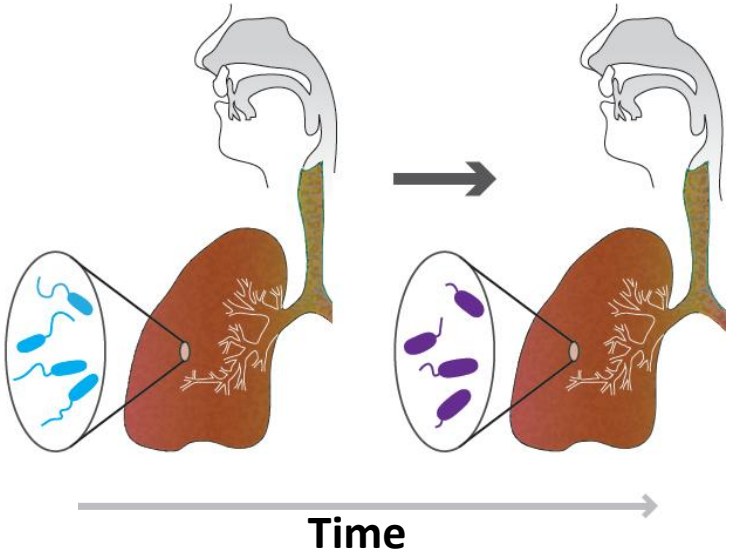
Epidemiology of cystic fibrosis patients can be monitored over time by genotyping bacterial isolates (each color indicate specific genotypes)



Jelsbak et al. (2007) Infect Immun

The *P. aeruginosa* DK2 clone type is a successful colonizer of the airways of cystic fibrosis patients

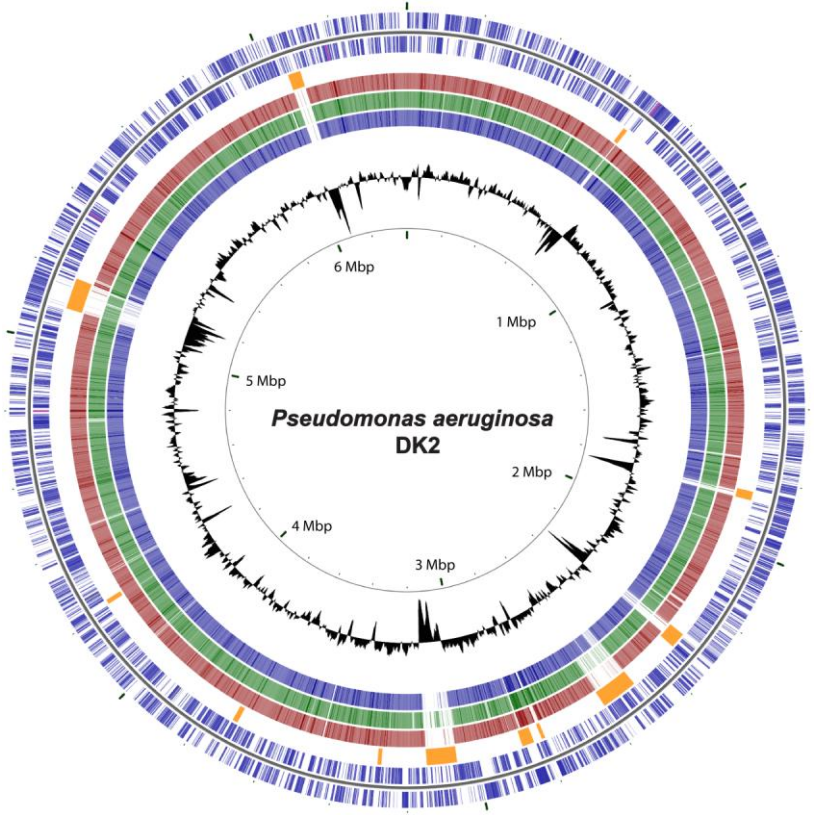
Genetic adaptation to host airways

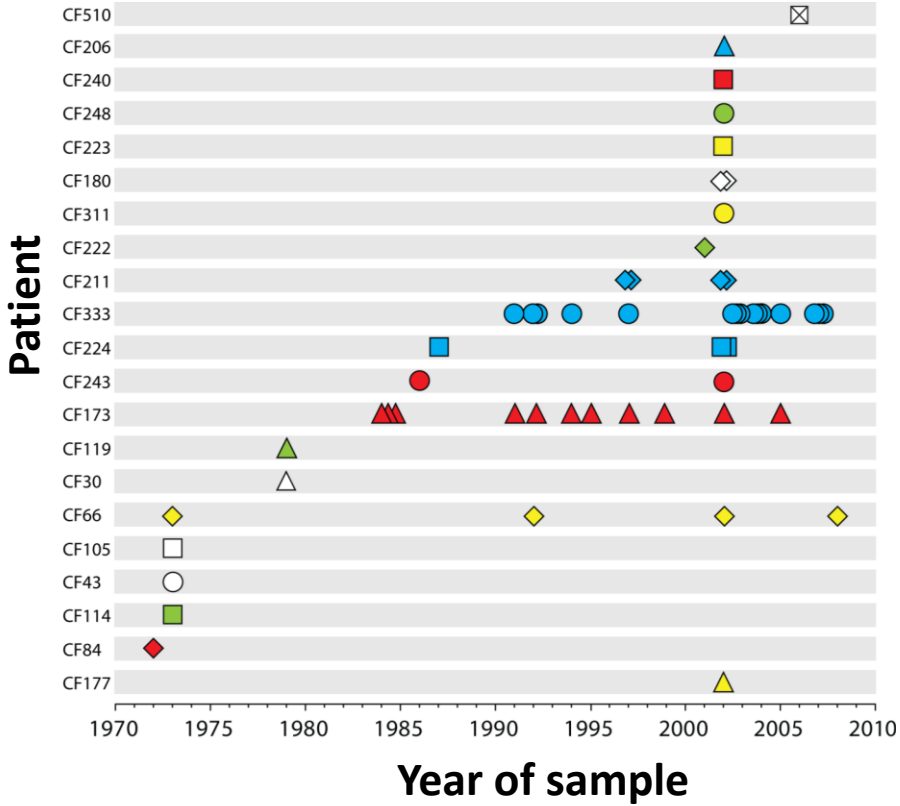


The DK2 clone has disseminated through a cohorte of more than 40 cystic fibrosis patients over a period of 38 years (≈200,000 bacterial generations)

Yang, Jelsbak, Marvig et al. (2011) PNAS

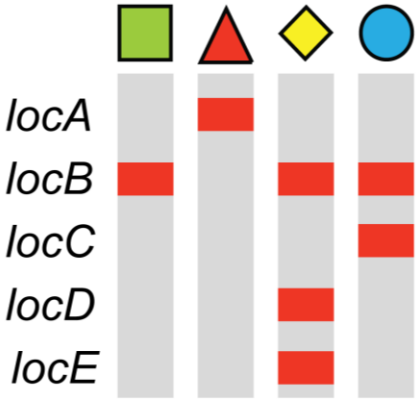
The genomes of 55 isolates of the DK2 clone type was sequenced to study the evolution of this bacterial pathogen





How predict the genetic relationship of clones?
Building amaximum-parsimonious phylogenetic tree

Genotype of clones



Genetic relationship

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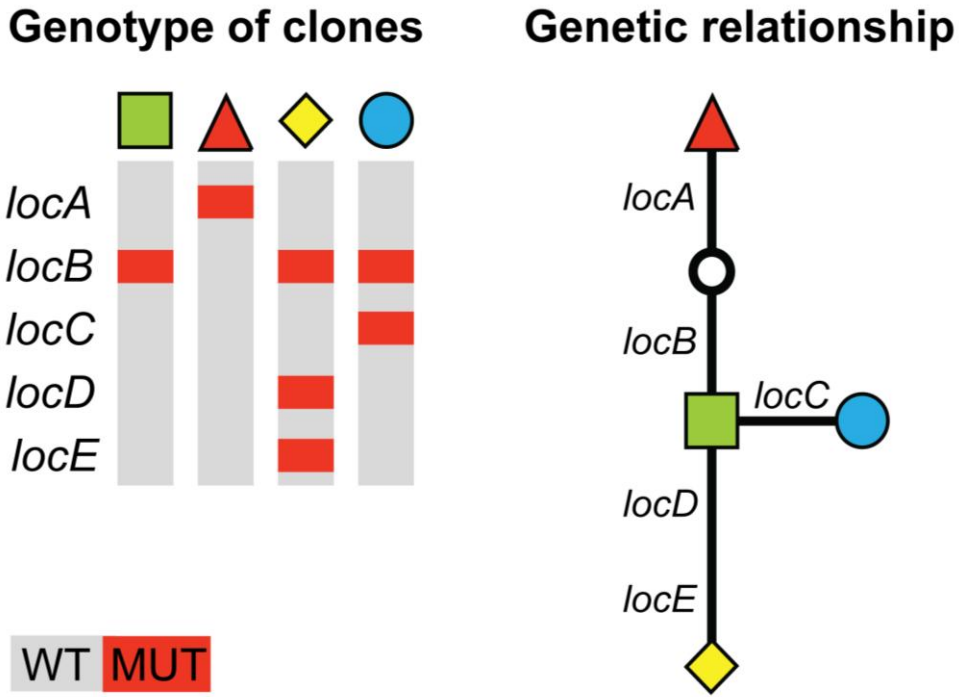
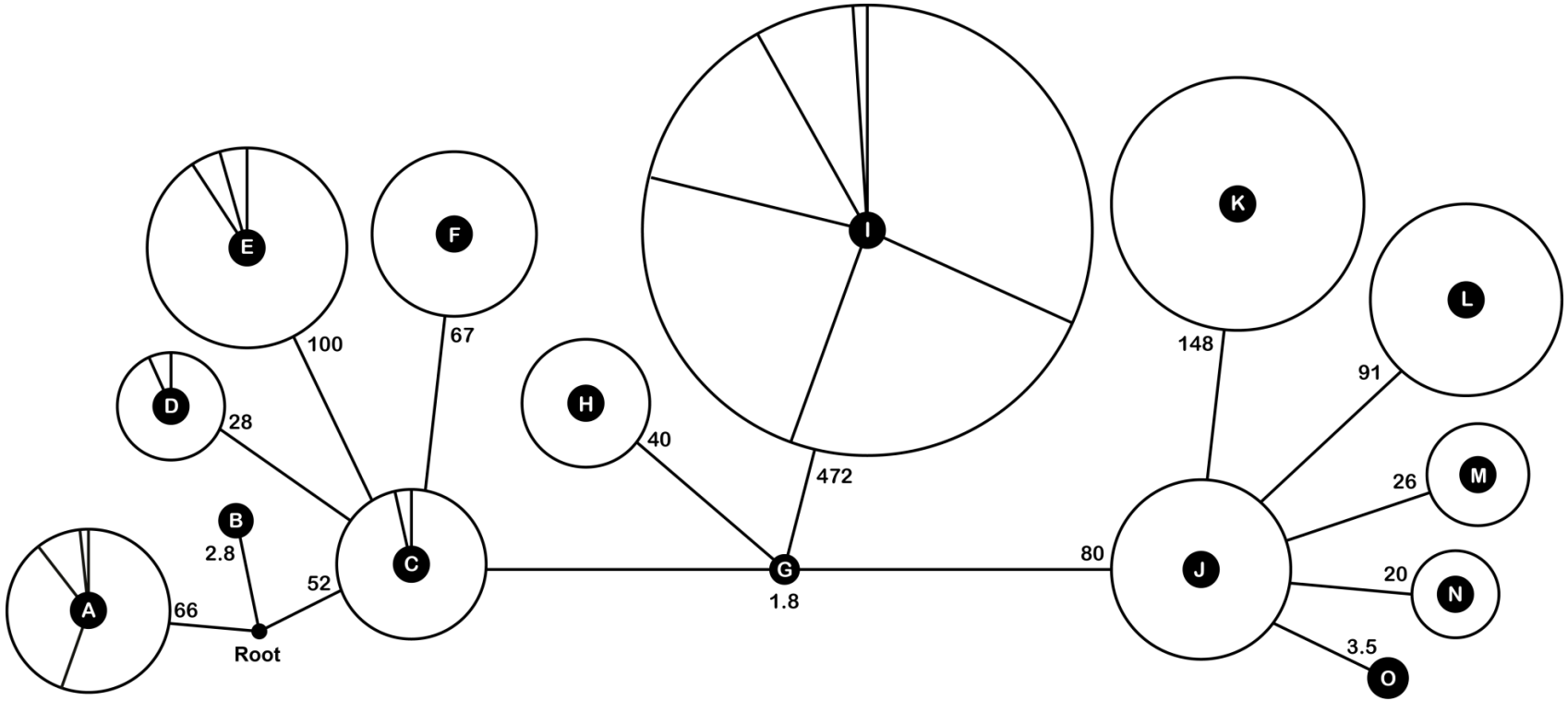
WT MUT

How predict the genetic relationship of clones?
Building a maximum-parsimonious phylogenetic tree

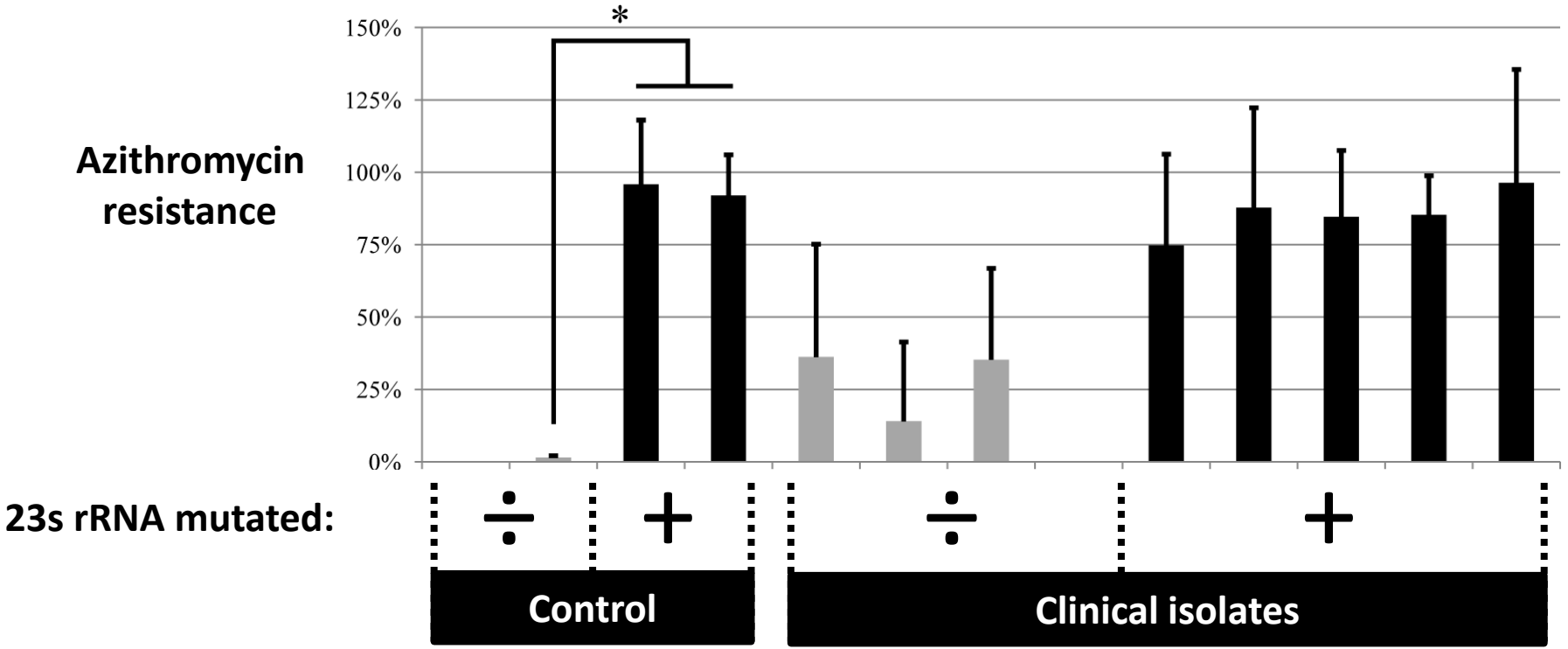


The DK2 genome is reduced during long-term infections (up to 8%)
No horizontal aquisition of novel DNA was observed



Rau, Marvig et al. (2012) Environ Microbiol

Case story - Parallel adaptive evolution: Multiple independent mutational events within the domain V of 23s rRNA



Marvig, Søndergaard et al. (2012) Antimicrob Agents Chemother

Conclusions

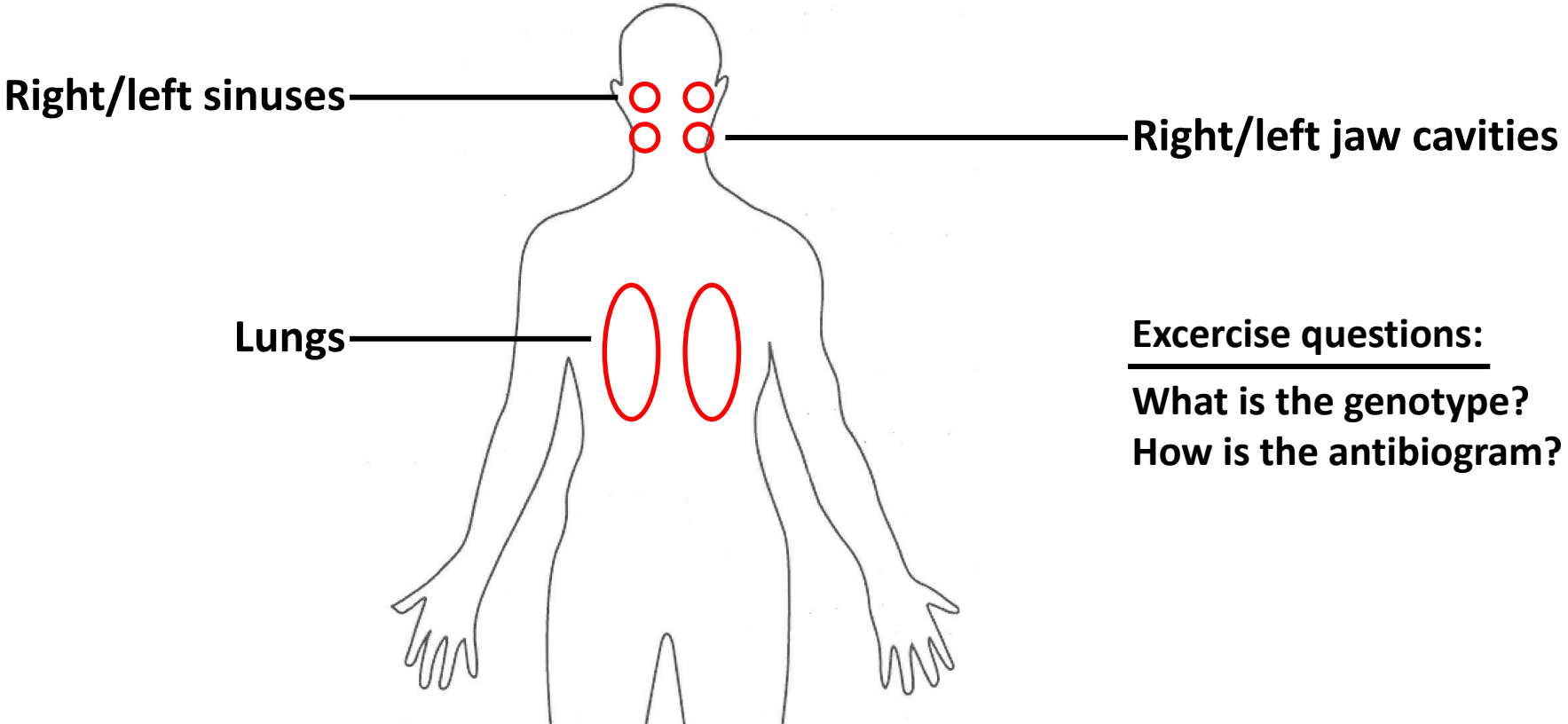
Genotyping of specific SNPs and variable genomics regions
can be used to identify the clone type of infecting bacteria (low resolution)

Whole-genome sequencing can detect genomic differences
between strains of the same clone type (high resolution)

Genotype-phenotype relationships can be established
by co-comparison of genotypes and phenotypes

Diagnostics Exercise Mission

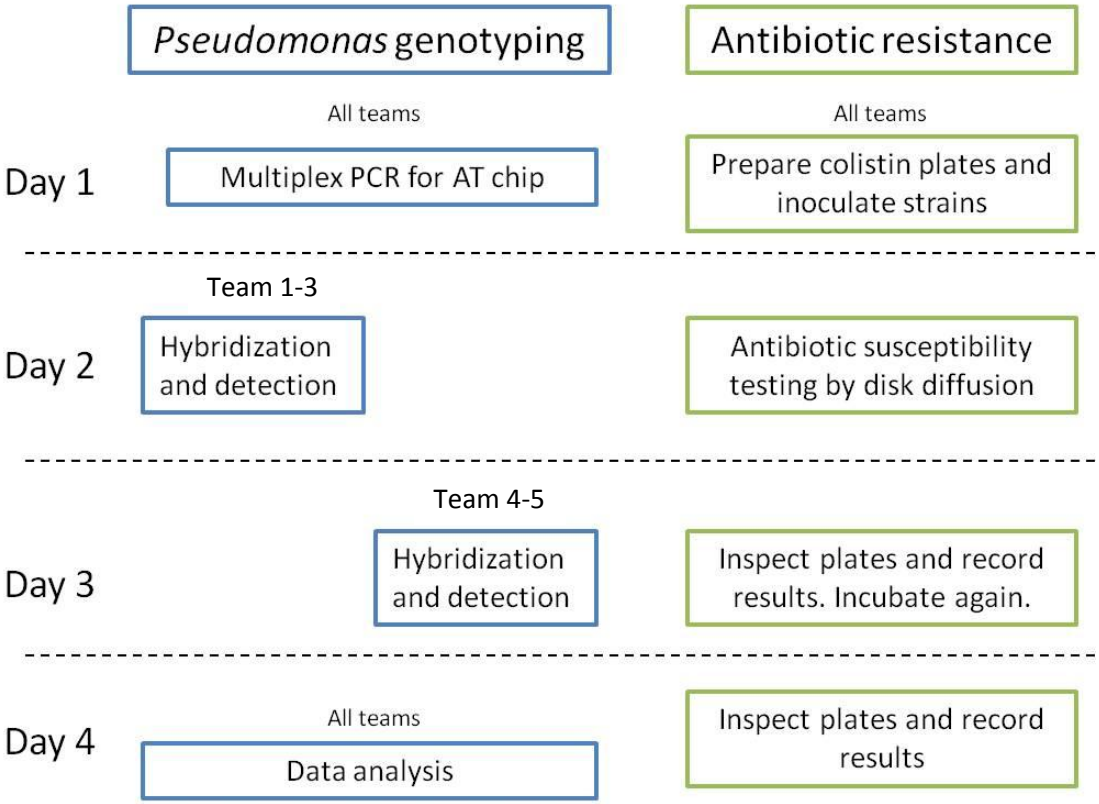
Patient suffering from chronic infections in sinuses, jaw and lungs
We need to make a microbial diagnosis of sampled *P. aeruginosa* clones



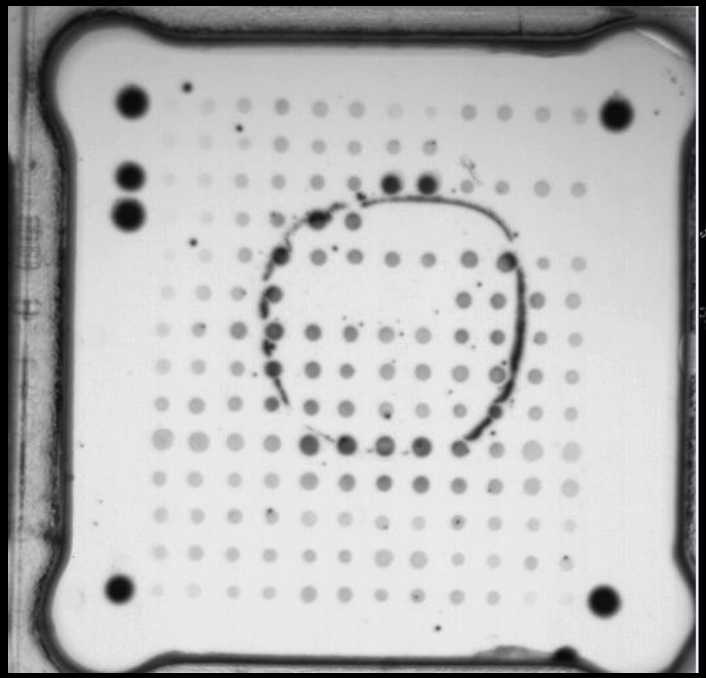
Excercise questions:

What is the genotype?
How is the antibiogram?

Overview of Microbial Diagnostics Exercise



Questions?



Acknowledgements

Infection Microbiology Group

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